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ON

10 JAN 2002

L1 82867 SEA AUTOLOGOUS (L) (LEUCOCYTE OR WBC OR WHITE BLOOD CELL OR WHITE BLOOD CELLS OR LYMPHOCYTE OR LEUCOCYTES OR LYMPHOCYTES OR LAK OR PAK OR LEUK!?)

L2 13872 SEA L1 AND (VIRAL OR VIRUS OR VIRUSES)

L3 957 SEA L2 AND (IL-2 OR INTERLEUKIN-2) AND (OTK OR OTK3 OR CD3)

L4 629 DUP REM L3 (328 DUPLICATES REMOVED)

L5 333 SEA L4 AND ANTI-CD3
D 1-33
D 11 IALL
D 34-300
D 218 KWIC
D 152 KWIC
D 152 IALL
D 130 KWIC

L6 1068 SEA L2 AND (IL-2 OR INTERLEUKIN-2) AND (OKT OR OKT3 OR CD3)

L7 716 DUP REM L6 (352 DUPLICATES REMOVED)

L8 333 SEA L7 AND (ANTI-CD3)
D 130
D 130 IALL
D 106 KWIC
D 106 IALL
D 68 IALL
D 10 IALL
D 7 IALL

L9 383 SEA L7 NOT L8

L10 190 SEA L8 AND HIV
D 1-190
D 190 IALL
D 189 IALL
D 161 KWIC
D 134 KWIC
D 99 KWIC

L11 205 SEA L9 AND HIV
D 1-205
D 205 IALL
D 204 IALL
D 126 KWIC
D 123 KWIC
D 122 KWIC
D 33 IALL

L12 178 SEA L9 NOT L11
D 1-178
D 178 IALL
D 177 IALL
D 78 IALL

D 72 IALL
D 71 IALL

L8 ANSWER 7 OF 333 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998178517 EMBASE

TITLE: HIV type 1-reactive chemokine-producing CD8+ and CD4+ cells

expanded from infected lymph nodes.

AUTHOR: Triozzi P.L.; Bresler H.S.; Aldrich W.A.

CORPORATE SOURCE: P.L. Triozzi, Ohio State University Medical Center, Division of Hematology and Oncology, N1011 Doan Hall, 410 W. 10th Ave., Columbus, OH 43210, United States

SOURCE: AIDS Research and Human Retroviruses, (20 May 1998) 14/8 (643-649).

Refs: 28

ISSN: 0889-2229 CODEN: ARHRE7

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 026 Immunology, Serology and Transplantation

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT:

The chemokines RANTES, MIP-1.alpha., and MIP-1.beta. have been identified as HIV- 1-suppressive factors produced by CD8+ T cells. We examined the possibility that HIV-1-specific, chemokine-releasing T cells could be expanded from the lymph nodes of patients with advanced infection. **Lymphocytes**, separated from lymph nodes of patients with peripheral blood CD4 counts less than 500/.mu.l obtained at diagnostic biopsies, were activated with ***anti*** -CD3 monoclonal antibody, and cultured in vitro for up to 12 days with IL-2. The phenotype, proliferative response, chemokine production, and anti-HIV-1 activity of the expanded cells was examined. Cells expanded 2.4- to 49-fold from patients with as few as 15 CD4+ cells/.mu.l in their peripheral blood. Expanded cells were a mixture of CD8+CD45RO+ and CD4+CD45RO+ T cells. The CD8+ cells were also CD30+CDw60+CD11b-. When challenged with **autologous** B cell targets expressing HIV-1 Env protein, unseparated expanded cells, and purified CD8+ and

CD4+ T cell subsets, proliferated and secreted MIP-1.alpha. and RANTES. Expanded cells were negative for HIV-1 by PCR and by culture. Culture supernatants inhibited the replication of HIV-1 in CD4+ cells in vitro. These studies indicate that HIV-1 can stimulate chemokine release by CD8+ and CD4+ cells expanded from infected lymph nodes, even from individuals with advanced infection. The numbers of chemokine-releasing T cells produced in these short-term cultures may be sufficient to be applied therapeutically as an ***autologous*** cellular therapy for HIV-1.

CONTROLLED TERM: Medical Descriptors:

*human immunodeficiency virus 1

*t lymphocyte subpopulation

*helper cell

*adoptive immunotherapy

immune response

lymph node biopsy

protein expression

cell proliferation

lymphocyte count

reverse transcription polymerase chain reaction

ACCESSION NUMBER: 97:379194 PROMT
TITLE: Conference Coverage (SPIRAT/NCDDG-HIV) Promising
Early Results from CD4 Reconstitution Trial
SOURCE: Vaccine Weekly, (7 Jul 1997) pp. N/A.
ISSN: 1074-2921.
LANGUAGE: English
WORD COUNT: 724
TEXT:

Two of the first three **HIV** infected patients to receive a new CD4 cell adoptive immunotherapy have sustained increases in their CD4(+) T-cell counts.

The new therapy, announced at the 1996 International Conference on AIDS and in a paper simultaneously published in the journal Science (see AIDS Weekly Plus, August 26/September 2, 1996), is based on the breakthrough finding that T cells

from **HIV** infected patients can be exponentially expanded in vivo by stimulation with beads coated with monoclonal antibodies to CD28 and ***CD3*** T-cell receptors.

"Normalization of numbers of circulating CD4 cells and CD4:CD8 ratios may be possible," said Carl H. **June**, head of the Immune Reconstitution Program at the Naval Medical Research Institute's Henry M. Jackson Foundation, Bethesda, Maryland.

June spoke in an address to "New Opportunities for **HIV** Therapy - From Discovery to Clinical Proof-of-Concept," the 2nd Joint Conference of the National Institute of Allergy and Infectious Diseases (NIAID)

Strategic Program for Innovative Research on AIDS Treatment (SPIRAT) and the National Cooperative Drug Discovery Groups for the Treatment of **HIV** Infection (NCDDG-HIV), held **June** 22-26, 1997, in Vienna, Virginia.

The rationale for CD4 cell adoptive immunotherapy is to repair the central action of **HIV** disease: disruption of the ability of CD4 T lymphocytes to regulate immune function.

The preliminary safety and feasibility study called for the three patients to receive increasing doses of autologous CD4 cells. The lymphocytes were collected by apheresis and expanded ex vivo via costimulation of their CD28 and

CD3 receptor molecules for 14 to 19 days. No antiretroviral drugs were added to the cultures.

Infusions of 3×10^9 , 1×10^{10} , and 3×10^{10} expanded cells were administered at six-week intervals.

Seven days after the last infusion, the patients had CD4 cell-count increases of 140 ± 26 , 109 ± 160 , and 311 ± 122 cells/(micro)L. These increases were sustained for more than four months in two of the patients, while the third had only transient increases.

"Results in [the latter] are consistent with blind homeostasis [of CD4 levels]," **June** said.

CD4 increases following infusion corresponded to increased dosages, but in a non-linear fashion.

Most importantly, there were no clinical toxicities other than mild chills and low-grade fever for less than 12 hours after the infusions. No increases in viral load could be detected by plasma **HIV-1** RNA assays.

"Assessment of the effects on immune function, T-cell receptor repertoire, and viral selection pressures is ongoing," **June** et al. wrote in their presentation abstract. "Together these findings suggest that manipulation of the CD28 signal transduction pathway has therapeutic potential for the treatment of **HIV-1** infection."

The ability to culture CD4 cells from patients with **HIV** infection is the culmination of seven years of work by **June**, Bruce L.

Levine, and colleagues. During efforts to stimulate growth of patients' cells, they created artificial antigen-presenting cells (APCs) by coating plastic beads with monoclonal antibodies capable of stimulating specific T-cell receptor molecules.

"This gives only an 'on' signal while normal APCs may give both 'on' and 'off' signals [depending on mitigating factors]," **June** said.

In 1996, **June** et al. made the surprising observation that CD28/***CD3*** stimulation not only caused the cells to proliferate but also made them resistant to **HIV** in the absence of antiretroviral drugs. Further investigation showed that induction of **HIV** resistance was specific to CD28 stimulation.

At the SPIRAT/NCDDG meeting, **June** noted that the antiviral resistance was specific for macrophage-tropic (M-tropic) **HIV** strains. These strains are responsible for most sexual transmission of the virus; individuals with inactivating mutations in the CCR5 chemokine receptor required by these ***HIV*** strains are highly resistant to infection.

"The CD28 antiviral effect is mediated by [CCR5] co-receptor downregulation and by enhanced beta-chemokine secretion," **June** said.

The findings suggest several approaches to the treatment of **HIV** disease:

- * Vaccines could induce the antibodies that mediate the anti-**HIV** and proliferative effects, thereby reversing the loss of CD4(+) T cells and inhibiting the spread of the virus.

- * Ex vivo proliferation of **HIV** resistant CD4(+) T cells could permit autologous replacement of cells killed by **HIV**.

- * The new cells expanded ex vivo could, prior to transfusion, be sensitized to

mediate immune responses against **HIV** or opportunistic pathogens.

* The newly expanded cells could provide immunologic help to CD8(+) T cells.

* CD4(+) lymphocytes altered by gene therapies could be expanded using the new technique. - by Daniel J. DeNoon, Senior Editor

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PRODUCT CODE: *PC8000223 Viral Disease R&D; PC8000430 Therapeutic
Procedures
EVENT CODE: *EC310 Science & research
INDUSTRY CLASS: *HLTH Healthcare - Medical and Health; BUSN Any type of
business
GEOGRAPHIC TERM: New: *CC1USA United States
Old: *CC1USA United States
FEATURES: NEWSLETTER

L8 ANSWER 68 OF 333 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1990:609418 CAPLUS

DOCUMENT NUMBER: 113:209418

TITLE: Long term expansion of cytomegalovirus-specific T cell

lines in the absence of antigen or antigen-presenting cells. Use of monosized polystyrene particles coated with agonistic antibodies

AUTHOR(S): Santamaria, Pere; Bryan, Mary Kay; Barbosa, Jose
CORPORATE SOURCE: Dep. Med., Univ. Minnesota, Minneapolis, MN, 55455, USA

SOURCE: J. Immunol. Methods (1990), 132(1), 1-11
CODEN: JIMMBG; ISSN: 0022-1759

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 15-1 (Immunochemistry)

ABSTRACT:

Functional and mol. studies of T **lymphocytes** involved in normal and abnormal immune responses, i.e., cells infiltrating tissues affected by autoimmune processes, require their previous in vitro expansion. Problems such

as unavailability of specific antigen(s) and/or the requirement of large amts. of **autologous** peripheral blood mononuclear cells (PBMNCs) as feeder cells, demand the development of alternative expansion methods.

Cytomegalovirus (CMV)-primed PBMNCs from several seropos. subjects were expanded for 5-6 wk by stimulation with **anti-CD3** coated onto polystyrene beads plus **interleukin-2 (IL-**

*****2*****) with a similar efficiency than when the MAb was presented by

*****autologous***** MNCs. Beads coated with anti-CD4, but not with anti-CD8, were also able to maintain the long term growth of CMV-primed populations of T cells. The expanded T cells of one of these polyclonal populations were cloned

by limiting diln. using **anti-CD3** or CMV, and

*****autologous***** PBMNCs as stimuli. Sixteen and 11 clones, resp., were obtained and grown to several million cells for 1-2 mo by weekly stimulations with **anti-CD3**-coated beads and **IL-2**.

Proliferation assays performed with most of the clones generated with

*****anti*** -CD3** stimulation showed that all the tested clones had retained the CMV and the class II MHC restriction specificities for at least 3-4 mo after the initial CMV stimulation. All the tested clones secreted

*****IL*** -2** in response to CMV and were **CD3+**, **CD4+**, and

CD8-. Comparison of the growth of 2 of these clones by stimulation with: (a)

*****anti*** -CD3**-coated beads and **IL-2**; (b)

*****anti*** -CD3, autologous** MNCs, and **IL-**

*****2*****; or (c) CMV, **autologous** MNCs, and **IL-2**,

showed that the first combination was at least as efficient as the other 2 in expanding these T cell clones. Thus, polystyrene monosized particles coated with agonistic antibodies can induce the long term growth of antigen-specific T

cell lines in the absence of specific antigen and feeder cells, often unavailable specially in the context of human autoimmune studies.

SUPPL. TERM: T lymphocyte culture antigen polystyrene particle

INDEX TERM: Antigens

ROLE: BIOL (Biological study)
 (T-lymphocytes specific for **viral**, long-term
 expansion of, antibody-coated polystyrene beads in)

INDEX TERM: Animal tissue culture
 (of cytomegalovirus-specific T-lymphocytes,
 antibody-coated polystyrene beads in long-term expansion
 of)

INDEX TERM: Antibodies
 ROLE: BIOL (Biological study)
 (to **CD3** antigen, polystyrene beads coated with,
 in long-term expansion of cytomegalovirus-specific
 T-lymphocytes)

INDEX TERM: Antigens
 ROLE: BIOL (Biological study)
 (**CD3**, antibodies to, polystyrene beads coated
 with, in long-term expansion of cytomegalovirus-specific
 T-lymphocytes)

INDEX TERM: Antigens
 ROLE: BIOL (Biological study)
 (CD4, antibodies to, polystyrene beads coated with, in
 long-term expansion of cytomegalovirus-specific
 T-lymphocytes)

INDEX TERM: Lymphocyte
 (T-, long-term expansion of cytomegalovirus-specific
 culture of, antibody-coated polystyrene beads in)

INDEX TERM: **Virus**, animal
 (human cytomegalo-, T-lymphocytes specific for,
 long-term
 expansion of, antibody-coated polystyrene beads in)

INDEX TERM: Lymphokines and Cytokines
 ROLE: BIOL (Biological study)
 (**interleukin 2**, antigen-specific
 T-lymphocyte cell lines dependent on, long-term
 expansion
 of, antibody-coated polystyrene beads in)

INDEX TERM: 9003-53-6D, Polystyrene, antibody-coated
 ROLE: BIOL (Biological study)
 (in long-term expansion of cytomegalovirus-specific
 T-lymphocytes)

cell culture
cytotoxicity
phenotype
disease control
human
human tissue
human cell
article
priority journal

Drug Descriptors:

*chemokine: EC, endogenous compound

*rantes: EC, endogenous compound

*macrophage inflammatory protein 1alpha: EC, endogenous
compound

*macrophage inflammatory protein 1beta

CAS REGISTRY NO.: (macrophage inflammatory protein 1beta) 122071-81-2

L10 ANSWER 189 OF 190 COPYRIGHT 2002 Gale Group

ACCESSION NUMBER: 96:277364 NLDB
TITLE: Conference Coverage (Vancouver AIDS Conference) CD4 T-Cell
Reconstitution in AIDS Now Feasible
SOURCE: Blood Weekly, (26 Aug 1996) .
ISSN: 1065-6073.
PUBLISHER: Charles W Henderson
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 1187
TEXT:
Long a goal of AIDS therapy, the reconstitution of lost CD4(+) T
lymphocytes may at last be nearing reality.

A breakthrough study shows that proper stimulation of CD4(+) T cells cultured from **HIV** infected individuals not only causes them to proliferate but also reduces **viral** load.

Moreover, the same stimulation of CD4(+) T cells from uninfected donors makes them resistant to **HIV** infection.

"We made the surprising observation that you didn't have to add antiretroviral drugs to the media to see this antiviral effect," said Carl H. June of the Naval Medical Research Institute, Bethesda, Maryland.

June announced the findings at the XI International Conference on AIDS, held July 7-12, 1996 in Vancouver, British Columbia, Canada, and in the journal Science ("Antiviral Effect and Ex Vivo CD4(+) T-Cell Proliferation in ***HIV*** Positive Patients as a Result of CD28 Costimulation," Levine et al., Science, 1996;272(5270):1939-1943).

The findings suggest several approaches to the treatment of **HIV** disease:

- * Vaccines could induce the antibodies that mediate the anti-**HIV** and proliferative effects, thereby reversing the loss of CD4(+) T cells and inhibiting the spread of the **virus**.

- * Ex vivo proliferation of **HIV** resistant CD4(+) T cells could permit ***autologous*** replacement of cells killed by **HIV**.

- * The new cells expanded ex vivo could, prior to transfusion, be sensitized to mediate immune responses against **HIV** or opportunistic pathogens.

- * The newly expanded cells could provide immunologic help to CD8(+) T cells.

- * CD4(+) **lymphocytes** altered by gene therapies could be expanded using the new technique.

A clinical trial is already underway, June said at the AIDS conference.

"We are just beginning to test growing CD4 cells this way in a clinical trial where cells will be grown ... and administered at increasing doses up to

3x10¹⁰ per patient to test the safety and feasibility of this approach," he said.

In their studies, June and colleagues set out to explore a previously reported phenomenon: the ability of CD28 signal transduction to prevent apoptosis in cultures of **HIV** infected cells.

In order for a T cell to become active after an antigenic molecule interacts with its major histocompatibility complex (MHC) receptors, it must receive other signals as well.

"The most important of the costimulatory signals identified to date is provided by the interaction of CD28 on T cells with its ligands CD80 and CD86 on antigen-presenting cells," noted Bruce L. Levine, June, et al. in their Science article.

To present a proper costimulatory signal to CD4(+) T cells, June and colleagues used monoclonal antibodies against CD28 and **CD3**. So that the antibodies would provide simultaneous signals, the researchers immobilized them on tiny beads.

June and colleagues took purified CD4 cells from the peripheral blood of a patient with intermediate-stage **HIV** disease and divided them into two cultures. One culture was activated with the anti-CD28/**anti-CD3** beads and the other was activated in the traditional manner using phytohemagglutinin (PHA) and **interleukin 2 (IL-2)**.

For the first 10 days, both cultures exhibited exponential growth. As is usual with CD4 cultures from people with **HIV** infection, growth in the PHA/**IL-2**-stimulated culture dropped off and the cells soon succumbed to the deadly effects of **HIV** infection.

But the cells stimulated with the immobilized CD28/**CD3** antibodies continued their exponential growth for 50 days.

"We showed that this could occur routinely in patients with intermediate stage **HIV** infection," June said, noting that similar results were obtained with CD4 cells from 10 **HIV**(+) individuals with CD4 counts ranging from 350 to 550 cells/(micro)L.

Cells in these experiments continued expanding for up to 70 days.

"The cultures were primarily CD4 cells," June said. "The average expansion in these 10 different cultures is 8,000 fold. The cultures weren't taken to termination but there was a wide variation between donors."

Viral DNA was measured at regular intervals in eight of the cultures. In all of these CD4 cells cultured from **HIV** infected donors, the RNA levels continually decreased. In most, the **viral** levels dropped to undetectable levels: that is, to less than five copies of **HIV** DNA per

100,000 cells.

To test the validity of this astonishing result, the researchers performed large-scale cultures of CD4(+) cells using three-liter bags and small-volume flasks. The cells in these studies came from an HIV(+) patient with a CD4 count of 393 cells/(micro)L.

At baseline, the cultures contained 800 copies of HIV DNA per 100,000 cells. Regardless of the culture conditions, viral DNA dropped to undetectable levels in all cases.

June and colleagues then wanted to see what would happen if they exposed CD4(+) T cells from normal, uninfected donors to the anti-CD28/anti-***CD3*** -coated beads and then exposed them to HIV.

Despite exposure to high infectious titers of HIV-1, the cells resisted infection. This resistance was shown to be specific for CD28 stimulation.

"CD28 costimulation conferred marked resistance to HIV-1 infection," Levine et al. wrote. "The effect did not appear to depend on the strain of ***virus*** used for infection, which is consistent with the ability of CD28 costimulation to increase the number of CD4(+) cells from multiple patients infected with HIV-1."

To explore the mechanism of this antiviral effect, the researchers exposed cultures of uninfected CD4(+) cells to various combinations of either soluble or bead-immobilized CD3 or CD28. The cultures were then exposed to infectious HIV-1.

There were three different outcomes:

- * In cultures exposed to soluble antibodies, there was rapid HIV infection and replication. No antiviral effect was seen.

- * In cultures exposed to immobilized antibodies, no HIV replication could be detected.

- * In cultures exposed to one immobilized and one soluble antibody, there were varying degrees of viral replication.

"We don't yet know the mechanism for this," June said. "We suspect it represents different cell-signalling pathways that are elicited depending on whether cells encounter unbound or immobilized antibody."

Interestingly, supernatants from six-day cultures of the costimulated CD4 cells had anti-HIV activity.

The recently recognized anti-HIV chemokines RANTES, MIP1(alpha), and MIP1(beta) could be detected in the supernatant, but when antibodies were added to neutralize the antibodies the anti-HIV effect remained. Moreover, stimulation conditions that enhanced virus replication also induced

chemokine secretion.

"We don't think the chemokines themselves are sufficient to explain the effect," June said.

Further studies showed that the anti-**HIV** activity affected an early stage of the **HIV** life cycle.

"It is a pre-integration event but post binding," June said.

There appears to be a window of opportunity for the antiviral effect.

"Preliminary results from a limited number of patients indicate that the antiviral effect may be less potent in late-stage **HIV** infection, even though CD28 costimulation still enhances CD4(+) T-cell proliferation," Levine et al. noted.

A high level of excitement was evident among researchers at the Vancouver AIDS conference.

"This is the most important paper I've seen in Science for years," enthused one researcher in response to June's presentation.

The scientists themselves are only slightly less restrained.

"Our results indicate that in vivo manipulation of CD28 interaction with B7 counterreceptors has the potential to enhance CD4(+) T-cell proliferation and prevent or limit **HIV-1 viral** spread in patients," Levine et al. wrote. - by Daniel J. DeNoon, Senior Editor

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CONTROLLED TERM: MH Medical and Health